

REMARKS

Claims 28-40 are pending. Claims 28-40 have been amended. Support for the amended claims exists throughout the specification, for example at page 61, Examples 9 and 10, Tables 4-8, Figures 7a, 8a. New claim 41 is supported, for example, at Examples 9 and 10, and Table 4. No new matter is introduced by these amendments and entry is requested respectfully.

Rejections under 35 U.S.C. § 112

On pages 2-3 of the Office Action mailed September 8, 2008, the Examiner rejects claims 29-40 under 35 U.S.C. § 112, second paragraph, as “being indefinite” for “relying on Examples and Figures in the specification.” Applicants respectfully traverse the rejection. Nevertheless, in an effort to expedite prosecution, the claims have been amended to recite directly the specific embodiments in the corresponding particular Tables and Figures. Hence, there is now clear correlation between immunoglobulin molecules described in the present application and those recited in the claims. Applicants respectfully request that these § 112, second paragraph, rejections be withdrawn.

On page 3 of the Office Action, the Examiner rejects claims 28-40 under 35 U.S.C. § 112, first paragraph, “for failing to comply with the written description requirement.” Applicants respectfully traverse the rejection. More specifically, the Examiner apparently questions whether one in the art would conclude that the method of claim 29 would encompass “one or more” immunoglobulins as recited in the claim in light of the specification which named thirty-three CD antigens. The Examiner similarly questions the descriptions of claims 30-34 by comparing the numbers of antigens listed in claims with numbers of antigens listed in the application tables and figures. Applicants have amended the claims to list the immunoglobulins that appear in the Tables and Figures previously recited in the claims, and delete language directing the reader to same. A reference to the immunoglobulins of the independent claim was also added to the dependent claims so that the Examiner may now count the markers in the claims, figures, and tables, and find that the numbers match.

Further regarding the claim amendments, the Examiner’s attention is invited to the specification (reference is made to the published application No. 20020019018) for an explanation of ‘kappa’, ‘lambda,’ ‘anti-hIg’ and ‘anti-Ig’:

[0219] The antibody array of this aspect of the present invention enables rapid and simultaneous detection of the expression of CD antigens on the surface of leukocytes isolated from blood. Antibodies against 51 CD antigens, 4 ***murine isotype control antibodies (IgG1, IgG2a, IgG2b, IgM)*** and antibodies against 7 ***other surface markers important in the diagnosis of leukemias*** (Glycophorin A, HLA-DR, KOR-SA3544, FMC7, ***immunoglobulin, kappa and lambda***) are attached as 5 nL dots to a film of nitrocellulose on microscope slides. Only leukocytes which express particular CD antigens bind to the corresponding antibody dot. ...

Additionally, the Examiner should recognize that the expression of kappa, lambda, and immunoglobulin (Ig) in leukemias are well known in the art. One of ordinary skill in the art would readily understand, in light of the knowledge at the time the application was filed, and the specification and figures, that for example, mIgG1 of Figure 7a refers to murine IgG1, and is used as a control; anti-hIg in Tables 5, 6 and 7, refers to human immunoglobulin; etc. Hence, there is correlation between immunoglobulin molecules described in the present specification and those recited in the claims, and adequate enablement and written description supports the inclusion of these immunoglobulins.

Moreover, the examples set out in Figures 7 and 8 in the present application show dot patterns for the human cell lines CCRF-CEM, NB4 and Raji (T-cells, myeloid cells and B-cells respectively). These examples show the different dot patterns (“patterns of binding”) that distinguish these different types of leukocytes.

Example 9 shows that the minimum array of antibodies for the present invention includes the use of CD3, CD4, CD8, CD14, CD19, and CD56. Further results can be obtained using one or more additional antibodies as disclosed on page 61, lines 22-23. Larger arrays can be produced to provide a wider screening opportunity as demonstrated in the Tables and results in greater precision of identification, or for analysis of other cell types. It is submitted that the Applicants are entitled to obtain patent protection for the invention in its broadest form that has been shown, or would be appreciated by a person skilled in the art, to work.

Also on page 3 of the Office Action, the Examiner rejects claims 28-33, asserting that “one of skill in the art would reasonably conclude that the application was not in position of the instant method utilizing derivatised supports beyond that of nitrocellulose coated glass...”

The present specification does, in fact, provide written description for a broad range of derivatised substrates:

[0122] The solid support is typically glass or *a polymer, such as but not limited to cellulose, ceramic material, nitrocellulose, polyacrylamide, nylon, polystyrene and its derivatives, polyvinylidene difluoride (PVDF), methacrylate and its derivatives, polyvinyl chloride or polypropylene*. Nitrocellulose is particularly useful and preferred in accordance with the present invention. A solid support may also be a hybrid such as a nitrocellulose film supported on a glass or polymer matrix. Reference to a “hybrid” includes reference to a *layered arrangement of two or more glass or polymer surfaces listed above*. The solid support may be in the form of a membrane or tubes, beads, discs or microplates, or any other surface suitable for conducting an assay. Binding processes to immobilize the molecules are well-known in the art and generally consist of covalently binding (e.g. cross linking) or physically adsorbing the molecules to the solid substrate.

[0215] The array of the present invention may also be adapted for use on a *microchip*. Microchip technology permits the generation of thousands of antibody patterns for a range of conditions and further permits automation and/or computer analysis. A “microchip” includes a *matrix support* comprising an array of adapter molecules, ligands or potential binding partners.

Hence, one of ordinary skill reading this paragraph would recognize that a solid support could be derivatised with a polymer, such as but not limited to cellulose, ceramic material, nitrocellulose, polyacrylamide, nylon, polystyrene and its derivatives, polyvinylidene difluoride (PVDF), methacrylate and its derivatives, polyvinyl chloride, or polypropylene. Additionally, new claim 41 recites the limitation “nitrocellulose film supported on glass,” hence this rejection should not be applied to the new claim. Applicants respectfully request that these § 112, first paragraph, rejections be withdrawn.

Rejections under 35 U.S.C. § 103

On page 4 of the Office Action, the Examiner rejects claims 28 and 29 under 35 U.S.C. § 103(a) “as being unpatentable over Gruber et al (Journal of Immunological Methods, 1993, Vol. 163, pp.173-179) in view of Wysocki and Sato (PNAS, 1978, Vol. 75, pp.2844-2848 ... and Delmarche et al (Science, 1997, Vol. 276, pp. 779-781).” Applicants respectfully traverse the rejection.

The present invention relates to a method for the identification of a type of leukaemia in a human subject. The pattern of interaction between the immunoglobulin molecules and the CD antigens in the sample is then determined, thereby providing an immunophenotype of the cells which is characteristic of the type of leukemia. More specifically, the claimed invention is a method

for distinguishing a leukemia of T cell, B cell, or myeloid lineage in a human subject by providing a single assay device comprising a derivatised solid support having an array of immunoglobulin molecules immobilized in discrete regions, wherein the immunoglobulin molecules are specific for the single cell surface marker antigens of CD3, CD4, CD8, CD14, CD19, and CD56, and wherein each immunoglobulin region specific for said single surface marker is present only once in the array; contacting a biological sample containing leukocytes with the assay device, wherein said biological sample is obtained from a human subject in need of a diagnosis of T cell, B cell, or myeloid lineage leukemia; allowing leukocytes in the biological sample to bind to the immunoglobulin molecules on the solid support via cell surface marker antigens on the leukocytes to form a pattern of binding on an array of discrete regions each being specific for a single cell surface marker presented only once in the array; and determining the relative scale of the pattern of simultaneous binding with which the cell surface marker antigens CD3, CD4, CD8, CD14, CD19, and CD56 on the leukocytes have bound to the immunoglobulin molecules on the array, wherein the relative scale of the pattern of binding on the array distinguishes leukemia of T cell, B cell, or myeloid lineage in the subject.

The Examiner asserts that “the phrase ‘determining the relative scale of the pattern of simultaneous binding with which the cell surface marker antigens CD3, CD4, CD8, CD14, CD19, and CD56 on the leukocytes have bound to the immunoglobulin molecules on the array, wherein the relative scale of the pattern of CD3, CD4, CD8, CD14, CD19, and CD56 binding on the array distinguishes leukemia of T cell, B cell, or myeloid lineage in the subject’” has no patentable weight “as it simply expresses a mental conclusion.” This language, however, does not relate solely to a mental step, as it may be conducted entirely by machine or by a combination of technologies. For example, the specification teaches that:

[0187] In a particularly preferred embodiment, the pattern of interaction is measured qualitatively or quantitatively as a pattern of density of either cells bound to the molecules in the array or cell-free antigens which have bound to the molecules of the array. ***The pattern of density may, for example, be determined macroscopically or microscopically or may be made with the aid of artificial intelligence such as using a computer guided densitometer. In addition, the present invention encompasses quantitation of binding pattern. Such computational aspects of the antibody arrays include, for example, numbers of cells, antigen- or cell-binding patterns, densities of binding numbers of cells per spot or numbers of antigens per cell.***

[0215] The array of the present invention may also be adapted for use on a microchip. **Microchip** technology permits the generation of thousands of antibody patterns for a range of conditions and further permits automation and/or computer analysis. A “microchip” includes a matrix support comprising an array of adapter molecules, ligands or potential binding partners.

[0226] The array is then observed microscopically, for example, using an Olympus BX60 fluorescence microscope (Olympus Optical Company, Japan), with a UPLan 4x objective with the condensor set at the phase 1 position and a green filter over the light source. **Images are captured and analyzed using a SenSys digital cooled CCD camera (1317 x 1035 Pixels, Photometrics), PCI Frame Grabber and Windows Image Processing and Analysis Software (Digital Optics). Images are processed for presentation using Adobe Photoshop version 3.0 software.** The intensities for specific binding of cells to each antibody dot is also recorded using a relative scale of +/-, +, ++, +++.

[0227] After **recording the results as computer files**, the antibody array bound cells are fixed by bathing the slide in FACS fixative (0.94% v/v formaldehyde, 2% D-glucose, 0.03% v/v NaN.sub.3, PBS (pH 7.3)) for 30 minutes. After rinsing in PBS, the bound cells are stained for 5 mins with Hemotoxylin counterstain (Immunotech, Marseille, France) which stains the nuclei of cells blue. The solid supports are then rinsed in PBS, dried and stored at room temperature. The solid supports may then be subsequently wetted with PBS and re-examined microscopically.

[0246] **The array is observed with an Olympus BX60 fluorescence microscope (Olympus Optical Company, Japan) using a UPLan 4x objective with the condensor set at the phase 1 position and a green filter over the light source. Images were captured and analyzed using a SenSys digital cooled CCD camera (1317 x 1035 Pixels, Photometrics), PCI Frame Grabber and Windows Image Processing and Analysis Software (Digital Optics). Images (6 antibody dots per frame) were processed for presentation using Adobe Photoshop version 3.0 software.** The intensities for specific binding of cells to each antibody dot is also recorded using a relative scale of +/-, +, +++++.

[0247] **After recording the results as computer files**, the antibody arrays with bound cells are fixed by bathing the slide in FACS fixative (0.94% v/v formaldehyde, 2% D-glucose, 0.03% v/v NaN.sub.3, PBS (pH 7.3)) for 30 mins. After rinsing in PBS, the bound cells are stained for 5 mins with Hemotoxylin counterstain (Immunotech, Marseille, France) which stains the nuclei of cells blue. Slides were rinsed in PBS, dried and stored at room temperature. The dried slides can be subsequently wetted with PBS and re-examined microscopically.

[0255] Using an array of monoclonal antibodies (e.g. against CD, MY or LY antigens), the **pattern of expression of particular antigens identified by this array is matched to set patterns of antigen expression for different leukemias (e.g. M4 AML (acute myeloid leukemia): MY4(CD14), MY7, MY9, MO1(CD11b)).** The cellular morphology provides a second criterion for diagnosis. **The diagnosis may be automated with fluorometric or spectrophotometric scanning of the arrays to determine which antibody spots bound cells, with computerized recognition of patterns of antigen expression for particular cancers.** This method enables **automated diagnosis** of a wide

variety of leukemias, lymphomas and other metastatic cancers. Using a complete array of antibodies against CD, MY or LY antigens, new types of leukemias and lymphomas may be discovered.

[0271] Wet slides are reviewed using dark-field microscopy at 400x magnification. Dots are photographed in groups of 6 and a composite picture produced using Adobe Photoshop software. ***Cell density on each antibody dot is quantified from the digital images using computer software or recorded by densitometric or fluorimetric scanning. The leukocytes bound on the array are stained by Alexa 488-conjugated anti-CD45 (leukocyte common antigen) and observed by fluorescence microscopy (FIG. 6). Three colour confocal microscopy is thus used for further identification and characterisation of cells bound to individual antibody dots.*** Histochemical stains (e.g. myeloperoxidase) is used on the cells bound to the arrays to confirm identification of some *leukemia* sub-types (e.g. AML).

Hence, there are many aspects to the claimed method that do not involve “mental conclusion,” but process steps analytically determined by instrumentation. Further, to the extent the method practices a mental selective step, that step is novel, nonobvious, and described in the specification as required by precedent. *Musco Corp. v. Qualite, Inc.*, 106 F.3d 427 (1997). Many a patentable invention rests upon its inventor’s knowledge of natural phenomena; many process patents seek to make abstract intellectual concepts workably concrete; and all conscious human action involves a mental process. *Lab. Corp. v. Metabolite Lab., Inc.*, 548 U.S. (2006). Thus, many processes are unquestionably patent-eligible, such as traditional industrial processes, processes that apply traditional life-science technology, processes for the treatment or cure of diseases, and the like, particularly where a method results in a useful, concrete, and tangible result.” *States St. Bank & Trust Co. v. Signature Fin. Group*, 149 F.3d 1368 (Fed. Cir. 1999).

Moreover, the claims have been amended to recite that the “biological sample is obtained from a human subject in need of a diagnosis of T cell, B cell, or myeloid lineage leukemia.” Hence, there is a clear limitation within the body of the claim that clarifies the claimed method applies to the diagnosis of leukemia in a human subject.

The claimed invention thus provides a pattern that provides a diagnosis in a single, rapid test, assisting clinicians to achieve a better understanding of their patient’s condition and thus provide better treatment. Since the filing of the present patent application, this approach has been applied to the analysis of over 700 patients and has proved over 90% accurate when compared to traditional techniques. Applicant’s award-winning technology has been also been commercialized in Australia. It is without doubt a useful and life-saving invention.

Comparisons between flow cytometry and the solid support array of antibodies according to the present invention were made and can be found on pages 64-65 and Table 8 of the specification. These results show the superiority of the present invention over flow cytometry (and other prior art tests). The Examiner's continued reliance on prior art relating to flow cytometry has not been substantiated or justified in raising obvious rejections of the present invention.

Returning to the rejection, the Examiner relies on the Gruber abstract which reads:

New fluorescent monoclonal antibody-dye conjugates permit three-color immunofluorescence analysis of leukocytes in whole blood using a single laser flow cytometer. The fluorochrome used in this study is a tandem conjugate of phycoerythrin (PE) and Cyan-5, which is excitable at 488 nm with a maximum in the emission spectrum at > 650 nm and it can be used together with PE and fluorescein isothiocyanate (FITC). The directly labelled monoclonal antibodies are incubated with unseparated anticoagulated blood and subsequently erythrocytes are lysed by a standardized automated procedure. The resulting leukocyte suspension can then be analyzed for three different surface markers in an individual sample of 100 microliters blood. When compared simultaneously with single-color analysis triple-color immunofluorescence yielded identical quantitative and qualitative results on various lymphocyte subpopulations. The efficacy of this method was evaluated by analyzing leukocytes of 42 healthy donors for the following markers: CD3, CD4, CD8, CD14, CD16, CD19, CD25, CD38, CD45RO, CD45RA, CD56, CD57, TCR-gamma/delta and HLA-DR. Of special interest was the finding that CD45RA and CD45RO are differently expressed in CD4 and CD8 cells. The reliability and convenience of this three-color analysis will make it possible to do more sophisticated examinations of subpopulations and their relevance in the monitoring of autoimmune diseases, immunodeficiency syndromes including AIDS and malignant disorders such as leukemias.

The Examiner asserts that Gruber supports an obviousness rejection because it "teaches the detection of the cell surface markers of CD3, CD4, CD8, CD14, CD19 and CD56, as well as CD16, CD25, CD38, CD45RO, CD45RA, CD57 and HLA-DR." Gruber discloses an assay using three-color flow cytometry to identify subsets of leukocytes: this technique is completely different from antibody microarrays. Flow cytometry utilizes fluid phase binding of antibodies to a cell surface, which is governed by mass action kinetics. The fundamental mechanism of action utilized by the present invention operates by physiologically based cell adhesion and rate kinetics to a solid surface and thus completely different to liquid phase cell interactions. Importantly, flow cytometry does not obtain the result in a single assay, but instead requires assembly of assays three-at-a-time, and hence multiple samples are required to achieve the same identification of subsets of leukocytes as the claimed invention.

Further Regarding claim 28, Gruber does not suggest, teach or disclose any subset or combination of the CD markers listed in the abstract for diagnosis of T cell, B cell, or myeloid lineage in a human subject as claimed by the present application. More specifically, there is no hint in Gruber that the claimed CD3, CD4, CD8, CD14, CD19 and CD56, as opposed to any other subset or combination of the fourteen markers listed the Gruber abstract, provides for the diagnosis of T cell, B cell, or myeloid lineage leukemia. Indeed, there are *at least eighty-four combinations* of panels of six markers that could be assembled from Gruber, including many containing *none* of the claimed markers. Moreover, as mentioned above, the method of Gruber allows for the testing of only three markers at a time: “The resulting leukocyte suspension can then be analyzed for three different surface markers in an individual sample of 100 microliters blood.” This greatly increases the number of possible combinations of assays that might be created to overlap with the claimed panel, with no suggestion as to which three markers would be more relevant than any other three for the diagnosis of leukemia.

The combination of Gruber, Wysocki, and Delmarche must suggest that this panel - CD3, CD4, CD8, CD14, CD19, and CD56 on the array - can be used to distinguish leukemia of T cell, B cell, or myeloid lineage in the subject in need of such diagnosis - in order to support an obviousness rejection. Clearly, Gruber does not.

On page 5 of the Office Action, the Examiner notes that Wysocki teaches that “lymphocytes from a heterogeneous population can bind to a solid support coated with an antibody specific for a cell surface antigen.”

Wysocki refers to cell-capture by a single immobilized antibody (“panning for lymphocytes”), and there is no extensive dot pattern for the identification of subsets of leukocytes. Wysocki discusses plastic dishes coated with Ig: “Polystyrene Petri dishes coated with rabbit anti-mouse Ig ...” (page 2845). Wysocki does not teach or suggest how these Petri dishes might be *derivatised*, as is the solid support of the instant claims. More importantly, Wysocki employed this method to *fractionate* and *separate* T and B lymphocytes (Abstract), and does not touch the characterization of these cells in relation to the diagnosis of a T cell, B cell or myeloid leukemia.

Wysocki does not suggest, teach, or disclose a complex cell surface phenotype for the identification of subsets of leukocytes. As such, Wysocki does not suggest, teach, or disclose the method for distinguishing a leukemia of T cell, B cell, or myeloid lineage in a human subject as presently claimed. The microarrays of immobilized CD antibodies that are used to capture

leukocytes expressing the complementary surface molecules (CD antigens) in Wysocki does not suggest, teach, or disclose the generation of extensive “patterns of recognition” or “disease signatures” of the claimed invention.

Also on page 5 of the Office Action, the Examiner cites Delmarche for the teaching of “a method of applying different immunoglobulins in a pattern on a elastomer coated solid support ...” Delmarche refers to schemes for using one or two different IgGs (mouse and chicken) in a micro-fluidic network and a relatively crude system for applying patterns of antibodies to a surface. Delmarche presents work related solely to immunoglobulin-immunoglobulin binding, and does *not* discuss cell-immunoglobulin binding such as the capture of live human cells. Delmarche does not suggest, teach or disclose extensive profiles of cell surface molecules or of mixed populations of proteins as a diagnostic tool. As with Gruber and Wysocki, Delmarche does not suggest using a specific panel of six markers (CD3, CD4, CD8, CD14, CD19, and CD56) for the characterization of cells in relation to the diagnosis of a T cell, B cell or myeloid leukemia.

In re O’Farrell, is instructive on the present rejection:

The admonition that “obvious to try” is not the standard has been directed mainly at two kinds of error. In some cases, *what would have been “obvious to try” would have been to vary all parameters or to try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many choices were likely to be successful.* ... In others what was “obvious to try” was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave *only general guidance* as to the *particular form* of the claimed invention or how to achieve it. *In re O’Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988) (emphasis added).

Gruber’s list of markers provides numerous possible choices of markers, with no indication of which parameters are critical or which of the myriad of choices are likely to provide for panel of six markers (CD3, CD4, CD8, CD14, CD19, and CD56) for the characterization of cells in relation to the diagnosis of a T cell, B cell or myeloid leukemia. The technologies of Wysocki and Delmarche provide, at best, only general guidance to the array of the present invention. By contrast, the present invention delivers a complex multiplexed phenotype for distinguishing a leukemia of T cell, B cell, or myeloid lineage in a human subject for which the combination of the cited art does not teach or suggest to the skilled person any more than do the individual documents. The presently claimed invention involves inventive step over any combination of the cited prior art. Hence,

because this § 103 rejection is inadequately supported by the cited references, Applicants respectfully request that it be withdrawn.

Moreover, the Court has instructed that objective evidence relevant to the issue of obviousness, i.e., secondary factors, *must* be evaluated by Office personnel. *Graham v. John Deere Co.*, 383 U.S. 1 (1966). Such evidence may include evidence of commercial success, long-felt but unsolved needs, failure of others, and unexpected results. Applicants previously provided the Examiner with a MEDSAIC press release, which reports that Applicants' licensee received the 2005 "BioFirst Commercialisation Award" for outstanding achievement in technology for its leukemia and lymphoma diagnostic. In addition to the recognition of commercial development and success in Australia, MEDSAIC was deemed most likely to achieve international success with its technology. This award evidences the recognition of others and commercial success of the claimed invention.

Applicants also previously provided the Examiner with a peer-reviewed article validating the Applicants application of the instant technology: Belov et al., "Analysis of Human Leukemias and Lymphomas Using Extensive Immunophenotypes from an Antibody Microarray," 135 British Journal of Haematology, 134-97 (2006). This paper clearly evidences the improvement over any of the techniques addressed in the art cited by the Examiner. In summary, comparing all of the cited references, in combination, to the claimed invention, it is clear that claimed invention reflects an advancement and "real innovation." *KSR Int'l. Co v. Teleflex Inc.*, No. 04-1350 (April 30, 2007) at 15. Hence, Applicants request that the § 103 rejections be withdrawn.

CONCLUSION

Applicants respectfully request reconsideration of this application and allowance of the pending claims in view of the above amendments and remarks.

Except for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 C.F.R. §§1.16 and 1.17 which may be required, including any required extension

of time fees, or credit any overpayment to Deposit Account No. 50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. §1.136(a)(3).

Respectfully submitted,

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